Formation of Bacterial Colonies in Successive Time Intervals

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By enumerating colonies on a plate in successive equal short intervals of time, we found that the number of colonies formed in individual intervals varied at random and their distribution was approximated by a Poisson series. Based on the result, we derived the equation of colony formation (CF equation). This equation describes the relationship between the cumulative number of colonies and incubation time: $N(t) = N_\infty\{1 - \exp[-\lambda(t - t_r)]\}$ where $N(t)$ is the number of colonies at time t. N_∞, λ, and t_r are parameters, expressing the expected number of colonies on a plate at infinite incubation time, the probability of the occurrence of colony formation in a unit of time, and a retardation time, respectively.

The number of bacterial colonies on a plate increases with incubation time. In spite of the same incubation conditions, in the case of purely cultivated bacteria, most of the colonies are formed on a plate after several days of incubation. However, in the case of soil bacteria, the number of colonies on a plate usually continues to increase even after a week. As a basic model describing the kinetics of bacterial colony formation on a plate, one of us (T. Hattori) proposed the equation of colony formation (CF equation), which was shown to be applicable to both purely cultivated and soil bacteria (3–6).

The CF equation contains three parameters, N_∞, t_r, and λ. The first parameter, N_∞, is the expected number of colonies on a plate at infinite incubation time. The second parameter, t_r, is the period of waiting time, which is the time lapse until the start of colony formation of the population on a plate; we call this parameter the retardation time. As discussed elsewhere (6a), the value of t_r consists of the time lag before the original population initiates proliferation and the time period during which the proliferating population starts to form colonies. After the lapse of time t_r, the event of colony formation of original cells occurs. The third parameter, λ, which characterizes the preceding event, is the probability of the occurrence of colony formation in regard to a certain cell of the population in a unit of time. And the inverse value of λ $(1/\lambda)$ is the mean time to the colony formation from time t_r; thus, the value $(1/\lambda) + t_r$ is the mean time to the colony formation from the start of incubation.

The interpretation of the CF equation implies a probabilistic concept of colony formation:bacteria on a solid medium form colonies by chance, and the probability of each colony formation in a unit of time is λ. This concept was proposed in the study of enrichment of soil bacteria (3): the proliferation of soil bacteria in capillary tubes was considered to occur through events with very small probabilities, even though the bacteria were dispersed into a medium. Hattori considered that the probabilistic concept of the proliferation of bacteria is not limited to microspace but is also applicable to a plate, and proposed the CF equation (3, 4).

The aim of this report is to confirm the probabilistic feature of bacterial colony formation on a plate directly and to present the theoretical derivation of the CF equation based on the experimental conclusions.

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MATERIALS AND METHODS

Organism. *Escherichia coli* IAM 1132 was supplied by the Institute of Applied Microbiology, Tokyo University, Japan. One loop of a preculture on conventional nutrient broth (NB medium) (2) was inoculated into 10 ml of the same medium, and after being kept at 27°C for 1 day, the culture was used as an inoculum for the plate count experiment.

Plate preparation. For making pour-mixed plates, NB medium was solidified with 1% Noble agar (Difco Laboratories, Detroit, Mich.). Plates were incubated in a thermostatic room at 27°C. After 12 h of incubation, we started the observations inside the room.

Design of observation. After the appearance of the first several colonies, we enumerated the colonies formed in 1-min (unit time) intervals by the naked eye, with the following specifications.

(i) The area of observation was restricted to the central part of a plate (4.5 cm in diameter). This part was almost uniform in depth, and the small colonies were easily observable.

(ii) The thermostatic room was kept dark enough to prevent any light reflection over the plate.

(iii) The Fujicolor Light Box 5000 (Fujicolor Trading Co., Ltd. Tokyo, Japan) was used as a light source. This apparatus gave light uniformly from the bottom of the plate. The plate was observed from an inclined position.

(iv) At 1-min intervals, we enumerated the colonies. Within each interval, we repeated the enumeration several times to be sure that we detected all new colonies. We did not enumerate doubtful ones which were detected on occasions by the successive checkings.

(v) Ten replicate experiments were carried out with various population levels. The period of observation varied from 40 to 60 min in accordance with the replicates.

RESULTS

Fluctuation of the number of colonies. When the number of colonies formed on a plate at the end of an experiment was relatively small, the colony formation seemed to occur sporadically (Fig. 1a). In one case, 17 colonies were formed in 50 min, i.e., the mean number of colonies formed in a unit time interval was 0.34. On an average, one colony formed every 3 min. But the length of the intervals varied from one interval to the next between 0 and 6 min. Besides, we could not find any regular interval of time between serial colony formations.
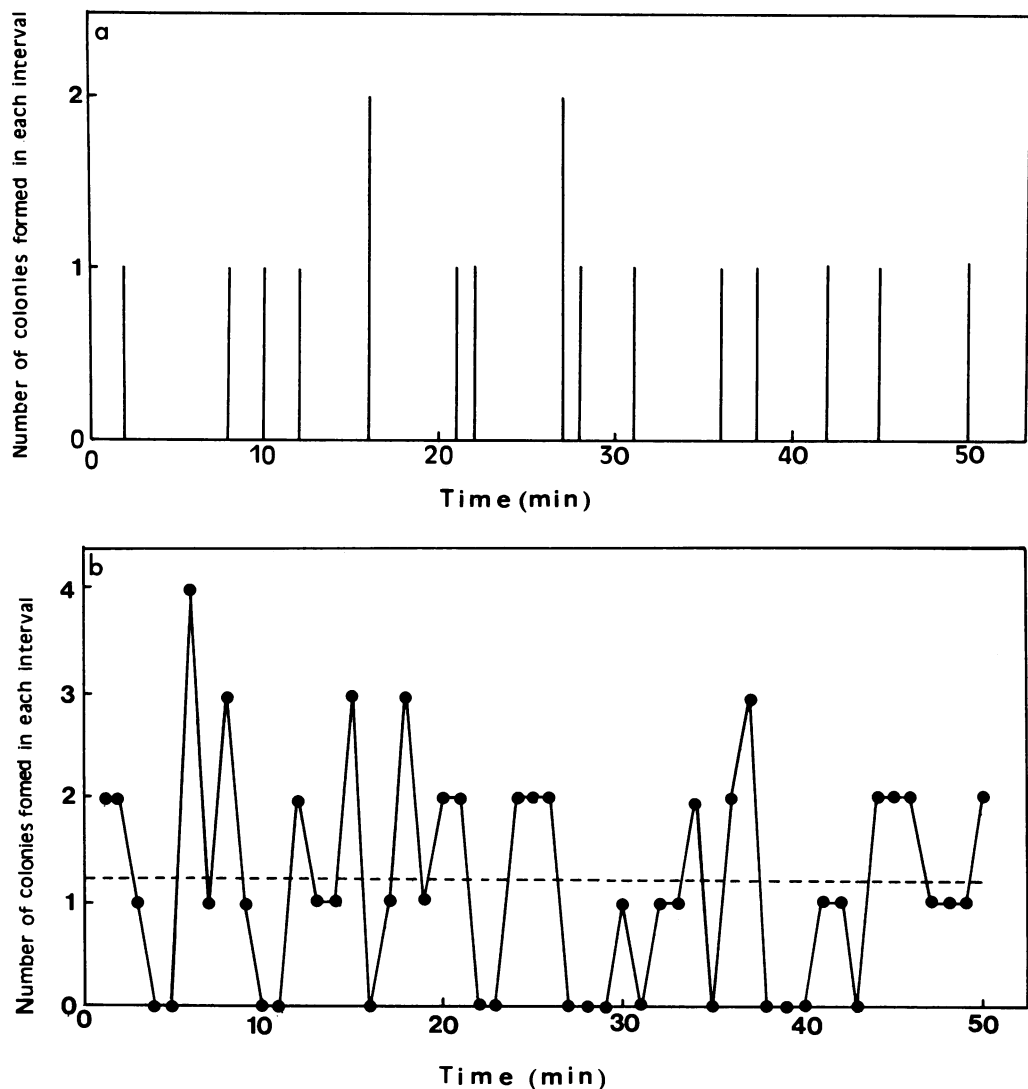
FIG. 1. Fluctuation of the number of colonies formed every minute, with the smaller population (a) and the larger population (b). In panel b, the dashed line shows the mean number.

Figure 1b shows the results of another replicate experiment. A total of 59 colonies were formed in 50 min. The numbers of colonies formed in individual time intervals varied from one interval to the next, like the fluctuation of the interval lengths between serial colony formations (Fig. 1a). The mean number of colonies formed in a unit time interval was 1.18, and the numbers of colonies formed in individual time intervals fluctuated around the mean number throughout the experiment.

Since essentially the same results were obtained throughout 10 replicate experiments with various population levels, the numbers of colonies formed in individual time intervals fluctuated at random, with no correlation relative to one another, apart from the fact that we obtained the mean number of colonies in a unit time interval. The numbers of colonies in individual time intervals fluctuated around the mean number. The frequency of obtaining larger numbers decreased as the number got further from the mean. It seemed that the numbers fluctuated following a kind of probabilistic distribution.

Distribution of the number of colonies. At first we calculated the variance of the numbers of colonies in unit time intervals. The obtained values from Fig. 1a and b were 0.311 and 1.089, respectively. In both cases, the values of the variances were similar to the mean numbers, 0.34 and 1.18, respectively. The good agreement between the values of variance and mean were consistent throughout 10 replicates (Table 1). The obtained numbers of colonies were whole numbers restricted to relatively small numbers between 0 and 4.

Based on these points, we considered that our results followed a Poisson distribution. As a statistical test for the goodness of fit between the obtained data and a Poisson distribution, we used the likelihood ratio test (10). In the case of Fig. 1a, the obtained χ^2-value was 42.225. Using the method of Wilson and Hilferty (11), we found out the probability that in a random sampling deviation systems as great as or greater than the obtained χ^2 value would arise. The obtained probability was 0.74, showing a good agreement with the Poisson distribution.

TABLE 1. Statistical values of 10 replicates[a]

Expt no.	Mean	Variance	χ^2 value	Degree of freedom	Probability
1	0.300	0.296	41.66	49	0.76
2[b]	0.340	0.311	42.23	49	0.74
3	0.420	0.371	44.75	49	0.65
4	0.500	0.378	42.98	49	0.72
5	0.580	0.493	48.23	49	0.50
6	0.600	0.583	62.78	59	0.34
7	0.800	0.779	44.10	39	0.26
8	0.975	0.794	40.11	39	0.42
9	1.067	1.216	79.47	59	0.04
10[c]	1.180	1.089	56.74	49	0.21

[a] The meaning of each statistical parameter is described in the text.
[b] Original data are illustrated in Fig. 1a.
[c] Original data are illustrated in Fig. 1b.

Table 1 shows the results of 10 replicates. As shown by the values of probability, the fitness between the obtained results and the Poisson distribution was quite good.

DISCUSSION

As described above, the numbers of colonies formed in individual time intervals seemed to fluctuate at random around the mean number. We think that this random fluctuation is caused by the fact that individual colony formations occurred with no correlation relative to one another, apart from the fact that on an average they occurred at some constant rate. We consider that the probability of the occurrence of colony formation of each cell in a unit time interval was constant throughout the experiment.

Based on the studies of α particles emission (9), we

derived the CF equation as follows: we set the parameter λ as the probability of the colony formation in a unit of time. Within a small time interval τ, $\lambda\tau$ (<1) colonies are expected to be formed. As described before, the distribution of the numbers of colonies is approximated by a Poisson series; the probability that no colony is formed within a small time interval from 0 to τ is

$$\frac{(\lambda\tau)^0}{0!} \exp(-\lambda\tau) = \exp(-\lambda\tau). \quad (1)$$

And the probability that a colony is formed within a further very small time interval $(d\tau)$ is

$$\lambda d\tau \quad (2)$$

The probability that no colony is formed within a small time interval from 0 to τ and a colony is formed within a following interval from τ to $\tau + d\tau$ is

$$\exp(-\lambda\tau) \cdot \lambda d\tau \quad (3)$$

By the integration of equation 3 from time 0 to time t, we obtain the probability of the formation of a colony until time t as follows:

$$\Phi(t) = \int_0^t \lambda \cdot \exp(-\lambda\tau) \cdot d\tau = 1 - \exp(-\lambda t) \quad (4)$$

Equation 4 corresponds to a certain cell. Actually on a plate, several cells form colonies independently with each other. We found in terms of parameter N_∞ the number of expected colonies at the infinite incubation time, and rewrote the equation as follows:

$$N(t) = N_\infty \cdot \Phi(t) = N_\infty[1 - \exp(-\lambda t)] \quad (5)$$

Setting the starting time of the incubation to time 0, we
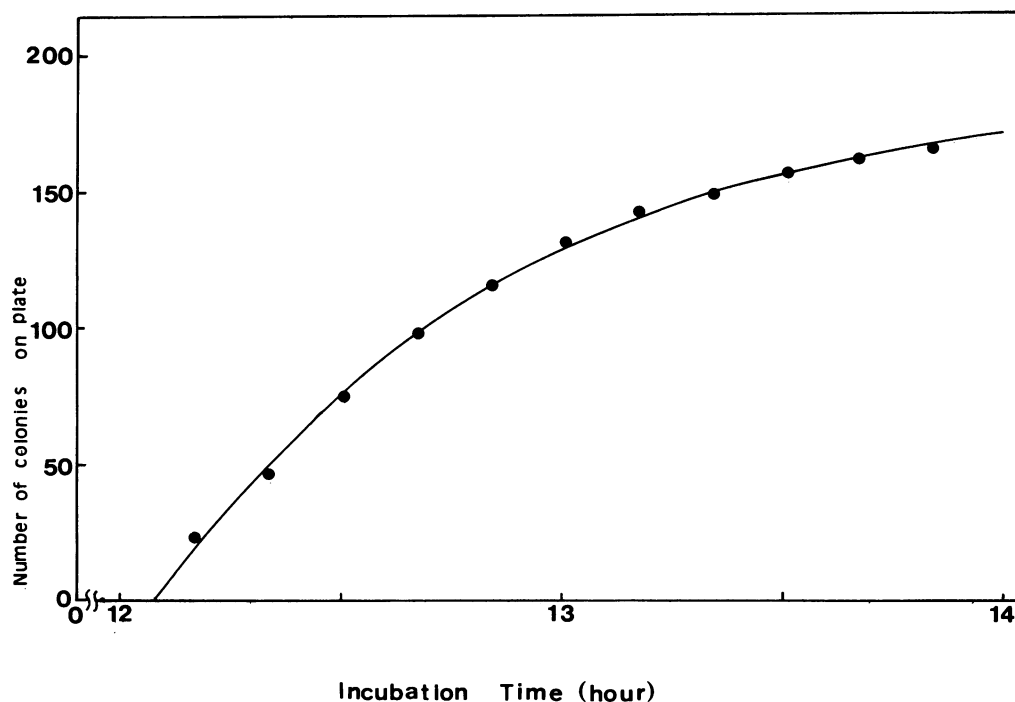

Incubation   Time   (hour)

FIG. 2. The application of the CF equation to the experimental results of the longer period of observation. Organisms and incubation conditions were the same as in Fig. 1, except for the population on a plate. The obtained parameters were $N_\infty = 191.455$, $\lambda = 29.540$ (day^{-1}), and $t_r = 0.503$ (day).

introduced the retardation time. This was because in the case of *E. coli* IAM 1132, it took 12 h from the start of incubation until the appearance of several colonies. We rewrote equation 5 with the new parameter describing a retardation time (t_r) as follows:

$$N(t) = \begin{cases} N_\infty\{1 - \exp[-\lambda(t - t_r)]\} & (t \geqq t_r) \\ 0 & (t < t_r) \end{cases} \quad (6)$$

Figure 2 shows an application of the CF equation to a set of plate count data of *E. coli*. The fitness between the CF equation and obtained result was quite good. Also in the plate count data of soil bacteria, the good fitness between this CF equation and obtained data has been reported (4, 5, 8). Thus, we consider the same random fluctuation might occur in soil bacteria, too.

The parameter λ varies with respect to the state of original cells: the λ values of an organism generally decreased as the culture became old or the cells were starved (4, 6). In the case of an oligotrophic bacterium isolated from soil, the λ values of freshly cultivated cells was higher than 1.0 day^{-1}, but when the cells were starved in deionized water for more than 3 weeks, the value decreased to lower than 0.5 day^{-1}. The preceding phenomena seem to correspond to the fact that the λ values for mixed populations in various soil samples were generally lower than 0.5 day^{-1} (4). From the standpoint of the CF equation we project that bacterial cells in soil are mostly in a state similar to that of starved cells.

When we enumerate the colonies, sometimes we may waver in our judgment on the recognition of colonies that contrast weakly with the medium. In the investigation of the detectability of light at the very threshold of vision, it is said that the detection of light is a probabilistic process; that is, in spite of flashes of the same intensity, sometimes the observer detects light and sometimes he does not (7). In the experiment of Hecht et al. (7), the flash duration time was only 0.001 s; thus, the observer could not double-check. However, we had enough time for several checks of the plate in a unit time interval. By these checks, we enumerated the colonies with confidence. In addition, the observations were carried out by one person in all 10 replicates; thus, the standard of colony detection was constant. We limited the probabilistic feature of the detectability to the greatest possible degree. We conclude that, as in the case of viruses attacking bacterial cells (1), the bacterial colony formation is satisfactory explained by a probabilistic approach.

The CF equation is important in many ways. For example, we can calculate the expected final number of colonies on a plate (N_∞) from several counts which are made in a relatively short period, and we can discover the physiological state of bacterial cells by means of the parameter λ. These problems will be discussed fully elsewhere (6a).

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